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BIOACTIVE/MEDICINAL COMPOUNDS

Effect of light quality and tissue origin on phenolic compound accumulation and antioxidant activity in *Camellia japonica* calli

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Abstract

In *Camellia japonica* callus culture, the effects of light quality on the accumulation of phenolic compounds were examined. To this end, the calli were cultured under three monochrome [white (W), red (R), and blue (B)] and two mixed [red/blue (RB) and red/green/blue (RGB)] light sources. After 4 weeks of culture, fresh and dry callus weights were determined, and phenolic content was analyzed using high-performance liquid chromatography. We also examined the effects of RGB light on calli derived from four origins (leaf: CL; root: CR; petal: CP; and ovary: CO). Total phenolic and flavonoid content and DPPH radical scavenging activity were highest in calli exposed to RB and RGB. Catechin was detected only in calli exposed to mixed light (RB and RGB). Interestingly, red pigmentation development in CL and CR calli after 2 weeks of culture and organogenic calli in CR and CP samples was observed. Furthermore, expression of the phenylalanine ammonia lyase and chalcone synthase genes was found to be higher in the CR line than in other lines, and total phenolic and flavonoid content was also higher in the CR and CP lines. The findings of the present study revealed that the accumulation of phenolics and flavonoids in callus tissue varies depending on light quality, which stimulates cell division, and may thus affect the proliferation and organogenesis of *C. japonica* callus. We also established that the effects of light on red pigment expression and the accumulation of bioactive compounds are dependent on the tissue origin of callus material.

Keywords Antioxidant activity · Callus culture · Mixed light sources · Phenolics · Flavonoids

Introduction

Camellia japonica (Theaceae) is an evergreen tree distributed in East Asia, where it is mainly grown for ornamental purposes (Li *et al.* 2016). The twigs, leave, and fruits of camellia contain catechin, epicatechin, cyanidin, and other bioactive compounds, and recent studies have demonstrated the antioxidant activity and efficacy of these compounds such as antiatherogenic effect (Lee *et al.* 2016; Chen *et al.* 2018; Páscoa *et al.* 2019). However, under field cultivation, the content of various *C. japonica* metabolites can differ markedly depending on the season, and the cultivation area is generally limited (Karuppusamy 2009; Li *et al.* 2016). To date, most of the studies that have examined the *in vitro* culture of *C. japonica* have tended to focus on regeneration *via* somatic embryogenesis (Vieitez *et al.* 1989; Vieitez and Barciela 1990; Barciela and Vieitez 1993). Different with *C. japonica* (camellia), many studies have been reported to produce useful bioactive substances in tea tree (*C. sinensis*) belonging to the same family with camellia (Zagoskina *et al.* 2003; Zagoskina *et al.* 2005; Wang *et al.* 2012). In particular, Zagoskina *et al.* (2003, 2005) reported that UV stimulates photosystem II (PSII) activity of phototrophic cells to increase photosynthesis-related photochemical activity when UV is irradiated under white light during the callus incubation period of *C. sinensis*.

Important among the range of strategies that can be employed to enhance the production of useful metabolites is the selection of callus lines containing high amounts of

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bioactive compounds, which is known to be influenced by variety, genotype, and explant source (Yue *et al.* 2016). For example, in *Tecoma stans* and *Linum usitatissimum*, it has been demonstrated that the content of total polyphenols and flavonoids can vary depending on the source of the explant from which callus is induced, with the highest amounts being detected in the callus induced from the stems of these plants (López-Laredo *et al.* 2009; Anjum *et al.* 2017).

Light quality is one of the critical factors affecting plant growth, development, and morphological characteristics (Fukuda *et al.* 2008), with light of different spectral properties stimulating different morphological and physiological responses (Batista *et al.* 2018). Although numerous studies have focused on the effect of light on photosynthesis, recent studies have reported that light also has an influence on the content of bioactive compounds (Miao *et al.* 2016; Llorente *et al.* 2017) and antioxidant enzyme activities (Carvalho *et al.* 2016; Qian *et al.* 2016; Yu *et al.* 2017) in plants including *in vitro* cultures (Bello-Bello *et al.* 2016; Pedroso *et al.* 2017). Light in the short wavelength region of the spectrum, such as blue and ultraviolet, has been shown to enhance the expression of genes associated with the flavonoid pathway and the content of bioactive compounds in a number of plant species, for example, blue light increased polyphenol contents and the total antioxidant status in lettuce (Johkan *et al.* 2010; Zoratti *et al.* 2014), and several phytochemicals and antioxidant enzyme activities in *Rehmannia glutinosa* (Manivannan *et al.* 2015). Furthermore, in the *in vitro* culture of cells and adventitious roots *of Eleutherococcus senticosus*, illumination with red light (660 nm) has been found to increase the content of bioactive compounds and activity of antioxidant enzymes (Shohael *et al.* 2006).

Although previous studies have examined the effects of light quality on the bioactive compound content of plants, few studies, with the exception of those on *Stevia rebaudiana* and *Prunella vulgaris* (Ahmad *et al.* 2016; Fazal *et al.* 2016), have focused on the effects of light quality on callus culture with respect to the production of useful metabolites. In stevia callus culture, blue light enhanced total phenolics and flavonoids; Ahmad *et al.* (2016) suggested that colored light can be promising to increase antioxidant secondary metabolites in callus cultures. However, to the best of our knowledge, there have been no studies that have investigated the production of secondary metabolites during the *in vitro* culture of camellia including callus and intact plants.

In this study, we investigated the effect of light quality on callus proliferation and the accumulation of phenolic compounds in camellia callus, and also examined the effect of explant origin on phenolic content and antioxidant activity in calli derived from four different explant sources.

Materials and Methods

Plant Materials Around 5-year-old *C. japonica* tree was grown in greenhouse, and for callus induction culture, four explants (leaf, root, petal, and ovary) were dissected from the mother tree. For the surface sterilization, explants were quickly immersed in 70% ethanol for several seconds, rinsed with sterile deionized water, and immersed in 2% (*v*/*v*) sodium hypochlorite solution supplemented with three to four drops of Tween-20 for 20 min. After three times rinse in sterile deionized water, explants were cultured on Murashige and Skoog (MS) medium (Murasnige and Skoog 1962) supplemented with 26.85 μM 1-naphthaleneacetic acid (NAA), 0.44 μM 6-benzylaminopurine (BA), 100 mg L^{-1} casein hydrolysate, 100 mg L^{-1} citric acid, 100 mg L^{-1} L-ascorbic acid, 30 g L^{-1} sucrose, and 2.4 g L^{-1} gelrite at 24 ± 1°C under dark conditions. The calli were maintained on the same medium for a year and used in this experiment. Masses of callus were plated in petri-dishes (five 250-mg masses per dish), and the four petri-dishes were cultured for the replication in each treatment. After 4 weeks of culture, the callus growth was measured with fresh and dry weight, and the organ (roots and/or shoots) emergence and pigmentation was examined under an SMZ-U stereomicroscope (Nikon, Japan). The organogenic callus was counted that the callus contains root or shoot primordia, and for the organ regeneration, the callus which produced shoots and/or roots longer than 3 mm. Those calli numbers were divided by the total inoculated calli mass per treatment and multiplied by 100 to represent percent data.

The content of phenolic compounds and activities of antioxidant compounds were analyzed. Additionally, we examined the expression of genes related with phenolic compound metabolic pathways in callus.

Light Quality To investigate the effect of light quality on the stimulation of secondary metabolite synthesis in *C. japonica* calli, we used LED light sources (PLCC 5450 6pin; Itswell Co., Incheon, Korea). At first, calli were placed under six different light sources: red (R), blue (B), red1/blue1 (RB), red1/green1/blue1 (RGB), white (W), and dark (D) for control. In the second experiment, 4 different origins of calli were placed and cultured under RGB. In both experiments, the wavelength of the light is as follows; R 645–675 nm, blue 440–460 nm, green 530–550 nm, and white 430–640 nm. The cultures maintained under around 65–75 μmol m^{-2} s^{-1} light intensity with a 16-h photoperiod at 24 ± 1°C for 4 weeks. Masses of callus were plated in petri-dishes (five 250-mg masses per dish), and the four petri-dishes were cultured for the replication in each treatment.

Flow Cytometry Cell division and DNA content was analyzed using flow cytometry. Samples used for analysis were prepared from freshly harvested callus (50-mg fresh weight) after

2 weeks of culture. Cell division was analyzed using a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA) after staining with propidium iodide (PI 05-5022; Sysmex, Lincolnshire, IL). For each cell cycle analysis, measurements were performed on at least three replicates. DNA index and cell division data were determined using the Cytexpert program (Beckman Coulter).

Analysis of Gene Expression Related to Phenolic Biosynthesis Aliquots of fresh callus (0.1 g) were collected from the culture medium and frozen with liquid nitrogen. Total RNAs from the samples were extracted using a RNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions, and treated with RNaseZap (Ambion, Austin, TX) to remove any residual genomic DNA. For cDNA synthesis, total RNAs were reverse transcribed using a ReverTra Ace qPCR RT Master Mix (Toyobo Co., Osaka, Japan). For real-time polymerase chain reaction (qPCR: CFX96 touch™ Real-Time qPCR Detection System; Bio-Rad Laboratories, Inc., Hercules, CA), we used 2 μL cDNA samples in conjunction with the specific primers (Table 1). The PCR conditions used to amplify the cDNA were as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 55°C for 20 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. For normalization, *C. sinensis* actin was used as an external standard, and the amplified DNA products were electrophoretically separated on a 1.5% agarose gel for visualization.

Analysis of Phenolic Compounds Extraction Samples of callus used for analysis of phenolic compounds were dried by freezing-drying in the light quality experiment and oven-dried in the RGB light experiment. The dried material was ground in a sterilized mulberry bowl, and 250-mg aliquots of the resulting powered dry calli were soaked in 20 mL 80% methanol at 24°C for 1 h with sonication (LS-2050-S10; LS-Tech, Gwangju, Korea). The sonicated material was then centrifuged twice at 10,000 rpm for 10 min each, and the collected supernatant was filtered through filter paper (Advantec, 110 mm, Tokyo, Japan; Toyo Roshi Kaisha Ltd., Tokyo, Japan).

Determination of Total Phenolic Compound Content Total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Folin and Ciocalteu 1927). Methanolic extracts (0.05 mL) were mixed with distilled water (2.55 mL), followed by the addition of Folin-Ciocalteu reagent (0.1 mL, 2 N). After 5 min, the reaction mixture was combined with 2.5 mL Na_2CO_3 solution (20%) and incubated in the dark at room temperature. The absorbance (change in color) at 760 nm after 30 min was measured using an Optizen POP spectrophotometer (Mecasys Co.). Concentrations were determined from a gallic acid standard curve (Sigma Chemical Co., St. Louis, MO), and total phenolic content was expressed in terms of milligrams of gallic acid equivalent per gram dry callus weight.

Determination of Total Flavonoid Content Total flavonoid content was determined using the colorimetric method described by Wu *et al.* (2006). Methanolic root extracts and a (+)-catechin standard (0.25 mL) (Sigma Chemical Co.) were prepared in 1.25 mL distilled water, to which was added 0.075 mL of 5% $NaNO_2$, followed by vigorous shaking. After 6 min, 0.15 mL of $AlCl_3$ solution (10%) was added to the sample, followed by incubation for 5 min at room temperature. Absorbance at 510 nm was measured using an Optizen POP spectrophotometer (Mecasys Co., Daejeon), and the results were expressed as milligrams of (+)-catechin equivalents per gram callus dry weight.

High-Performance Liquid Chromatography Determination of Individual Phenolic Compounds Samples of powdered callus material (0.25 g) were sonicated (SD-250H, SD-ULTRASONIC CO., Seoul, Korea) for 1 h in 80% methanol to ensure complete extraction. The extract was filtered through filter paper (Advantec, 110 mm), and the solvent was evaporated. The dried residue was dissolved in 10% methanol and fractionated twice with 10 mL diethyl-ether/ethyl-acetate (1:1) prior to evaporation to dryness under. The residues of both fractions were combined and dissolved in methanol prior to filtration through a membrane filter (0.2-μm pore size; Whatman, Maidstone, UK). A photodiode array (PDA)–equipped HPLC system (2690 Separations Module, Waters Chromatography, Milford, MA) was used to analyze the

Table 1. Sequences of *Camellia japonica*–specific primers for PCR

Gene	Accession number	Primer	Primer sequence (5′ to 3′)	Amplicon size (bp)
Actin	HQ235647.1	Forward Reverse	TTACGGAACGTGGCTACTCC GCTGCTTCCATTCCAATCAT	238
CjPAL	JQ860361.1	Forward Reverse	GCTGAGCAACACAACCAAGA GCAAAACCTTGAAGGGTGAA	240
CjCHS	AB512766.1	Forward Reverse	CGTGAAGTGGGCCTTACATT GCCCGTAGCTTCTCTTCCTT	203

phenolic compounds. Separation was performed using a Fortis C18 column (5 μL, 150 × 4.6 mm). Acetonitrile (A) and 0.1% aqueous acetic acid (*v*/*v*) (B) were used as the mobile phase, with a linear gradient of 8–10% A at 0–2 min, 10–30% A at 2–27 min, 30–90% A at 27–50 min, 90–100% A at 50–51 min, 100% A at 51–60 min, and 100–8% A at 60–70 min. The column was re-equilibrated for 10 min between injections at a 1.0 mL min^{-1} flow rate, and 20-μL aliquots were injected into the HPLC for each analysis. Calibration plots were obtained by measuring the peak areas. UV absorption spectra and retention times were used as criteria for the identification of individual compounds.

Analysis of Free Radical Scavenging (DPPH Activity) The antioxidant capacity of callus phenolics was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH: Sigma Chemical Co.) method described by Hatano *et al.* (1998), with some modification. DPPH radical solution (0.8 mL 200 μM DPPH) was added to 0.2 mL of methanolic sample extract, with a 40% methanol solution serving as a control. The solution thus prepared was incubated for 5 min at room temperature, and absorbance at 517 nm was measured using an Optizen POP spectrophotometer (Mecasys Co.). The scavenging percentage for the antioxidant capacity was calculated as follows:

$$\%Scavenging = \left[\left(A_{control\ absorbance} - A_{sample\ absorbance}\right)/A_{control\ absorbance}\right] \times 100$$

Statistical Analysis For the statistical analysis, one-way analysis of variance (ANOVA) was used to determine the significance of differences among the mean of groups. Statistical assessments of the difference between mean values were then performed using Duncan's multiple range test. A *P* value of 0.05 was considered to indicate statistical significance, and all data were analyzed using SAS software (SA 9.4; SAS Institute Inc., Cary, NC).

Results and Discussion

Effect of Light Quality on Cell Proliferation and Phenolic Compounds After 4 weeks of callus culture under the five different light treatments, the highest callus fresh weight was obtained in the dark control treatment (D) (Fig. 1). Among the different light treatments, callus fresh weight was higher in material exposed to monochrome R (1.20 g·$mass^{-1}$) and mixed RB (1.15 g·$mass^{-1}$) illumination than in calli subjected to other light treatments, which represented 2.1- and 2.0-fold increases, respectively, compared with the white light control treatment (W). With the exception of treatment D, dry weight was also highest in callus exposed to red light, followed by RB, RGB, and B. These observations are consistent with the findings of previous studies, which have reported that that red light (610–760 nm) is effective in promoting biomass accumulation, shoot elongation, root development, and organ regeneration in different species (Sivakumar *et al.* 2006; Daud *et al.* 2013; Kwon *et al.* 2015). Crosstalk between red light and cytokinin activity has also been described by To *et al.* (2004), who demonstrated the involvement of the A-type *Arabidopsis* response regulator (ARR) 4 gene in this process. Similarly, Osakabe *et al.* (2002) found that cytokinin-inducible genes were also overexpressed in plants overexpressing the A-type ARR gene in *Arabidopsis*. Moreover, increased expression of A-type ARR4 has been observed to promote cell division and increase shoot formation (Osakabe *et al.* 2002; Riefler *et al.* 2006).

In the present study, we also detected the initial development of red pigmentation on the surface of callus after 15 d of culture under R, RB, and particularly RGB light (Fig. 1*b*). Red light has similarly been reported to be involved in red pigment accumulation in strawberries and peas (Wu *et al.* 2007; Miao *et al.* 2016), whereas numerous studies have also reported that red pigment is expressed when plants are exposure in blue light (Johkan *et al.* 2010; Kadomura-Ishikawa *et al.* 2013; Xu *et al.* 2014),

The total phenol and flavonoid content of calli was investigated after 4 weeks of photoperiod treatment (Fig. 2*a*). We found that the total phenolic content was highest in calli subjected to the RB mixed light treatment [9.06 mg g^{-1} dry weight (DW)], followed by the RGB mixed light treatment (6.03 mg g^{-1} DW) and control (dark) (5.21 mg g^{-1} DW). In all calli exposed to the different light treatments, total flavonoid content was higher than in the control calli, with levels being the highest in those exposed to RB (4.87 mg g^{-1} DW) and RGB (4.45 mg g^{-1} DW) light.

Our analysis of the DPPH radical scavenging activity in calli revealed that the highest activity (82.4%) was obtained in calli exposed to RB mixed light (Fig. 2*b*). The activity was higher under RGB mixed light treatment (58.4%) and red light (34.1%) than that in calli cultured in the dark (30.5%), whereas the lowest DPPH radical scavenging activities were detected in calli cultured under single white (23.2%) and blue (25.0%) lights.

When plants are exposed to light, reactive oxygen species (ROS) are produced *via* the activity of cytochrome P450, particularly in response to illumination with blue light (Darko *et al.* 2014; Lobiuc *et al.* 2017). Furthermore, in a study on cucumber, Wang *et al.* (2010) detected increases in the levels of H_2O_2 and salicylic acid and the expression level of phenylalanine ammonia-lyase (PAL) and polyphenoloxidase in response to red light irradiation, and that plants show short- and long-term responses associated with the elimination of ROS, which involves the production of phenolics such as caffeic

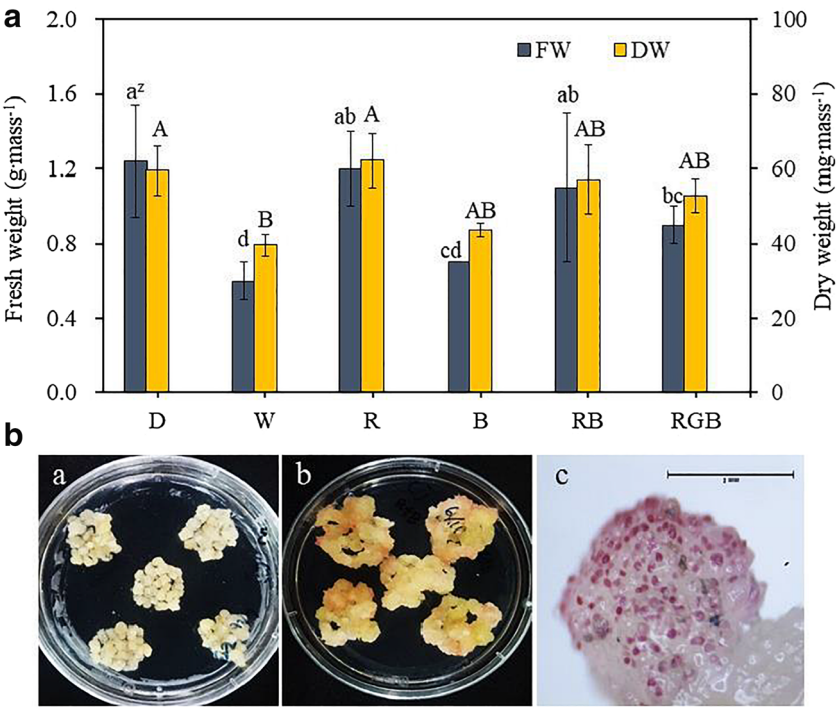

Figure 1. Effects of light quality on callus proliferation in *C. japonica*. (*a*) Fresh and dry weight (D: dark, W: white, R: red, B: blue, RB: red1/blue1, RGB: red1/green1/blue). (*b*) Callus after 4 weeks of culture. (*a*) W, (*b*) RGB, and (*c*) red pigment expression under RGB (*white scale bar* = 1 cm, *black scale bar* = 1 mm). *Different letters* indicate significant differences at $P < 0.05$ according to Duncan's multiple range test ($n = 10$)

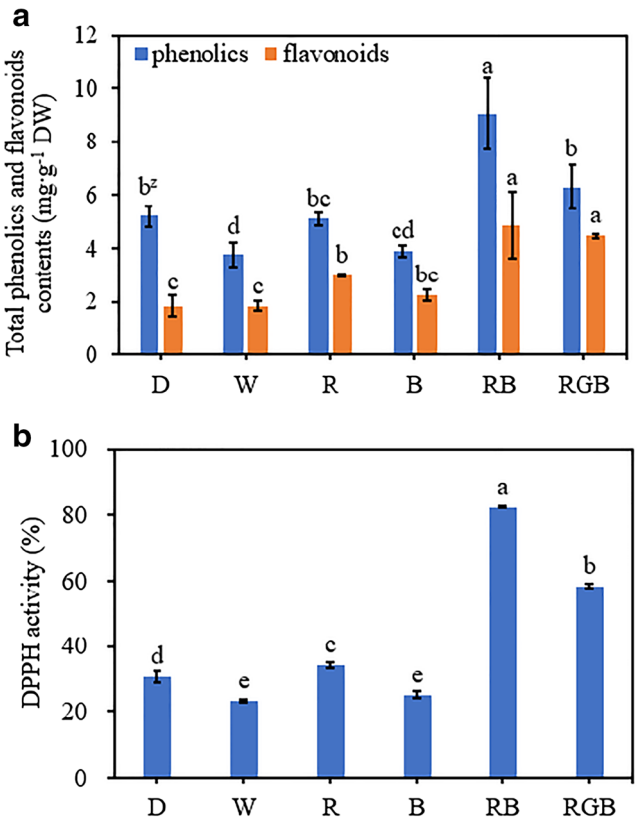

Figure 2. Effect of light quality on content of total phenolics and flavonoids in *C. japonica* calli after 4 wk of culture. (***a***) Content of total phenolics and flavonoids. (***b***) Free radical scavenging activity determined using DPPH (D: dark, W: white, R: red, B: blue, RB: red1/blue1, RGB: red1/green1/blue). *Different letters* indicate significant differences at $P < 0.05$ according to Duncan's multiple range test ($n = 4$).

acid and rosmarinic acid (Lobiuc *et al.* 2017). Batista *et al.* (2018) reported that blue light plays a role in regulating microtubule synthesis-related genes and secondary metabolite synthesis-related genes, and that red light and green light are also closely associated to the expression of defense-related genes. Moreover, in lettuce, blue light has been demonstrated to enhance the content of antioxidant polyphenols, anthocyanins, and carotenoids (Li and Kubota 2009; Johkan *et al.* 2010), whereas Li and Kubota (2009) reported an increase in phenolic compounds in lettuce illuminated with red light.

On the basis of the findings of these studies, we can therefore assume that camellia callus produces larger amounts of phenolics when exposed RB and RGB, as a consequence of defense-related mechanisms.

Characteristics of Calli Cultured from Explants Derived from Multiple Origins Under RGB Light After 4 weeks of culture under RGB light, higher callus fresh weight was obtained in the three lines derived from root, petal, and ovary tissue (CR, CP, and CO), without any significant difference, whereas callus derived from leave material (CL) had a comparably lower fresh weight. Conversely, along with CR, dry weight was found to be higher in CL calli (77.5–78.3 mg·mass^{-1}) (Fig. 3), and these calli were also observed to be characterized by high organ differentiation. Interestingly, we observed the most pronounced development of red pigmentation (70%) on the surfaces of CL and CR calli with the highest dry weights (Fig. 4). In contrast, CR (66.7%) and CP (93.3%) lines were characterized by predominantly greenish calli.

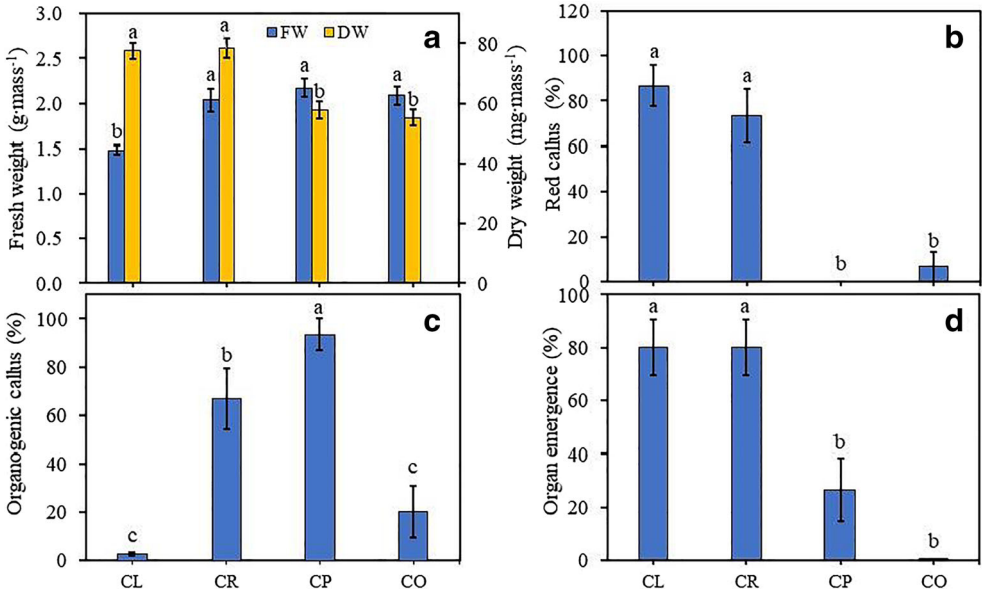

Figure 3. Effect of RGB (red1/green1/blue1) light on proliferation of *C. japonica* calli derived from 4 different origins after 4 wk of culture. (***a***) Fresh and dry weight ($n = 10$). (***b***) Percentage of red pigmented calli ($n = 4$). (***c***) Percentage of organogenic callus ($n = 4$). (***d***) Organ regeneration (CL leaf-induced callus, CR root-induced callus, CP petal-induced callus, CO ovary-induced callus) ($n = 4$). *Different letters* indicate significant differences at $P < 0.05$ according to Duncan's multiple range test.

Given that the levels or responses of endogenous hormones are often organ dependent, the source of explants is considered to be an important factor with respect to callus proliferation and organogenesis (Piovan *et al.* 2010). In this regard, different levels of endogenous auxin in calli derived from different organs may influence pigment accumulation in callus under different light conditions, and Ji *et al.* (2015) reported that anthocyanin, one of the red pigments, accumulates *via* the transcription of anthocyanin biosynthesis-related genes in response to a reduction in auxin content. Moreover, it has been reported that stem-derived callus accumulates larger amounts of anthocyanins than leaf-derived callus in *Crataegus sinaica* (Maharik *et al.* 2009). Therefore, we proposed that the observed differences in pigment expression can be attributed to differences in the auxin sensitivity of callus derived from different origins.

In the present study, we observed that of the calli derived from four explants' different origins, CP calli showed a high percentage of cells in the G_2M phase of the cell cycle (Fig. 5*a*), which indirectly supports our assumption that CP was an organogenic callus, as shown in Fig. 3, as usually organogenic callus contains meristematic cells. We also

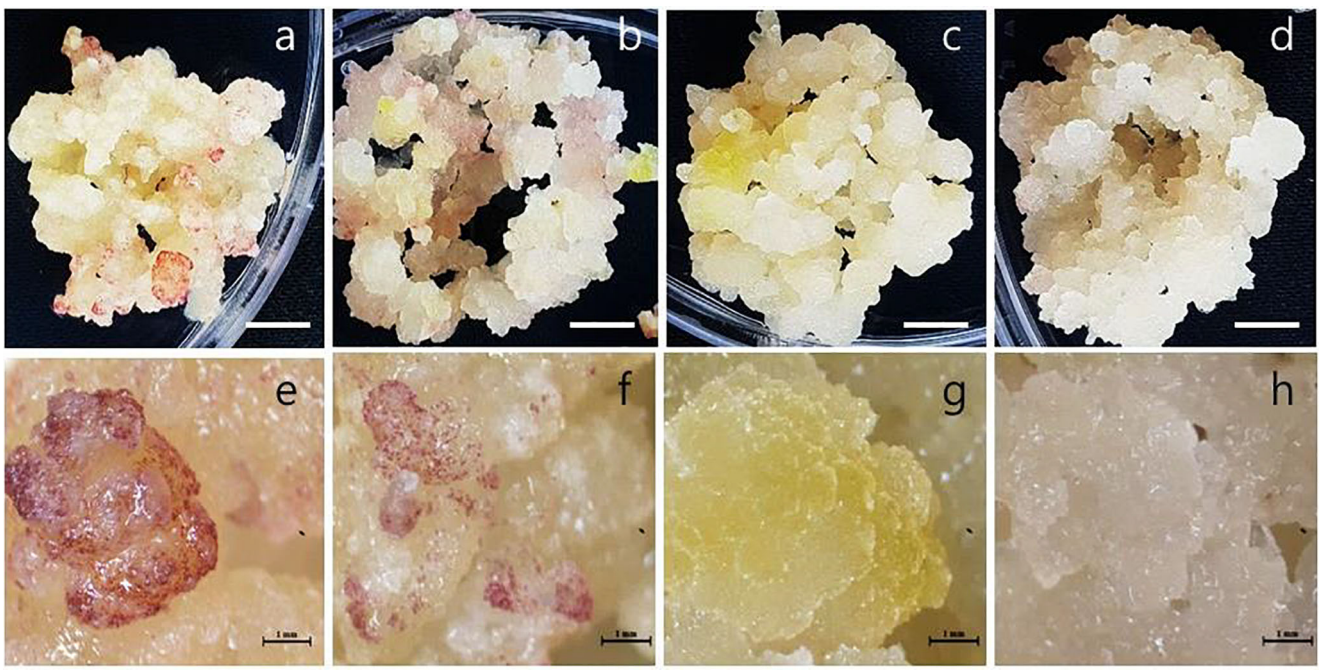

Figure 4. Effect of RGB (red1/green1/blue1) light on pigmentation in *C. japonica* calli derived from 4 different tissue origins. (***a***, ***e***) Leaf-derived callus (CL). (***b***, ***f***) Root-derived callus (CR). (***c***, ***g***) Petal-derived callus (CP). (***d***, ***h***) Ovary-derived callus (CO). *White scale bar* = 1 cm, *black scale bar* = 1 mm.

detected variations in DNA content depending on callus origin (Fig. 5b), with the other three lines showing values ranging between 1.7 and 1.9 when the index value of the CP line was set at 1.0. (data not shown). On the basis of these observations, we suspect that somaclonal variation (SV) may have occurred in these latter three callus cultures, presumably as a consequence of repetitive subcultures. SV is characterized by both genetic and epigenetic variations (Rodríguez López *et al.* 2010), the former of which influences DNA replication and cell division and can result in altered ploidy, chromosomal rearrangement, and changes in specific sequences (Wang and Wang 2012). Therefore, we assumed that cell division was most active in CP callus, which did not show any evidence of genetic SV.

Analysis of the expression of *PAL* and chalcone synthase (*CHS*), two enzymes involved in the phenylalanine pathway, in the calli derived from four different tissues revealed highest expression levels in the CR line (Fig. 6). The PAL and CHS enzymes play roles in a pathway-associated phenolic and flavonoid biosynthesis (Slatnar *et al.* 2010), and higher expression levels of *PAL* and *CHS* in rose leaves have been reported to result in an increased biosynthesis of phenolics (Shetty *et al.* 2011). The transcription of *PAL* and *CHS* is regulated by endogenous circadian rhythms, which in turn have been shown to be affected by environmental conditions such as light (Thain *et al.* 2002), and particularly exposure to red and blue light (Feinbaum *et al.* 1991; Batschauer *et al.* 1996; Ahn *et al.* 2015). In this regard, Bordage *et al.* (2016) reported

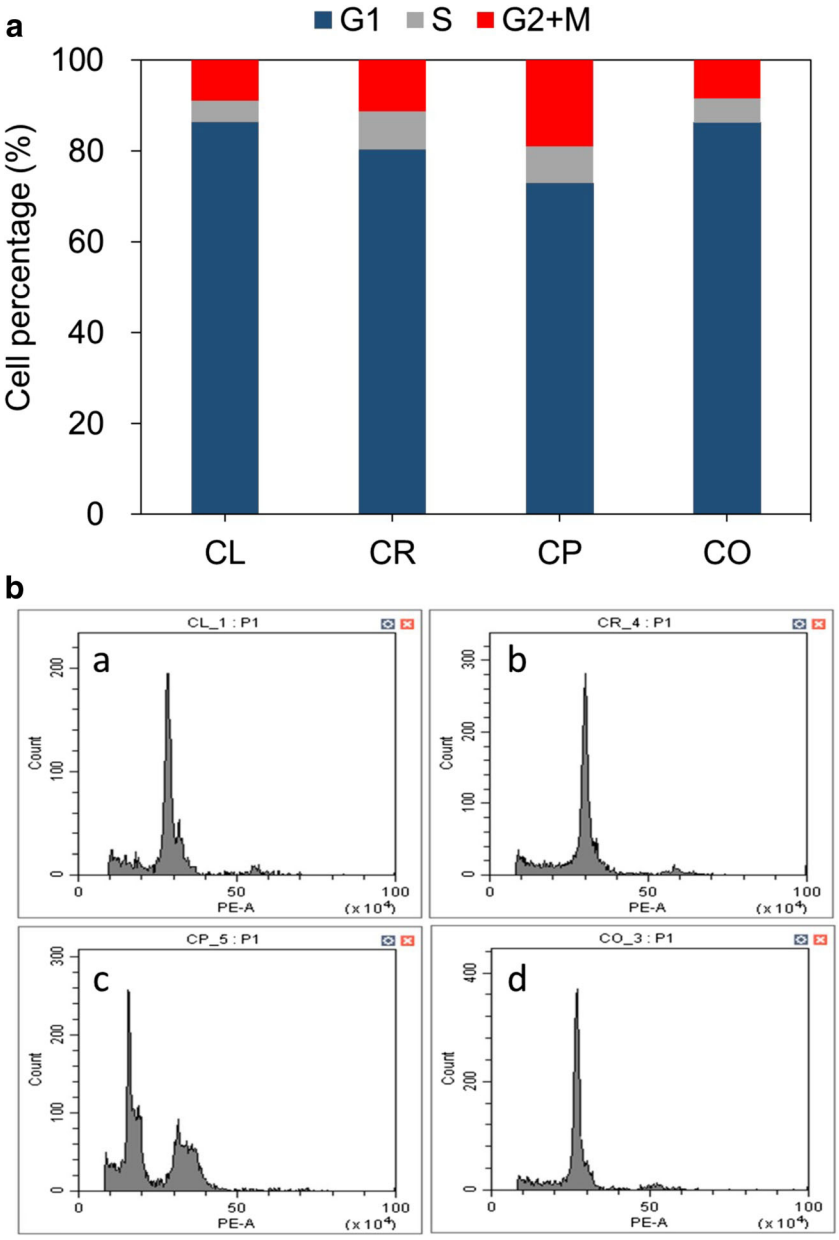

Figure 5. Effect of RGB (red1/green1/blue) light on cell division in *C. japonica* calli derived from 4 different tissue origins after 4 wk of culture. (***a***) Cell cycle of calli (G1-Gap1 stage, S-DNA synthesis, G2 + M-Gap2 and metaphase). (***b***) Histogram of DNA content in calli of 4 different tissue origins. (*a*) CL: leaf-derived callus, (*b*) CR: root-derived callus, (*c*) CP: petal derived callus, and (*d*) CO: ovary-derived callus.

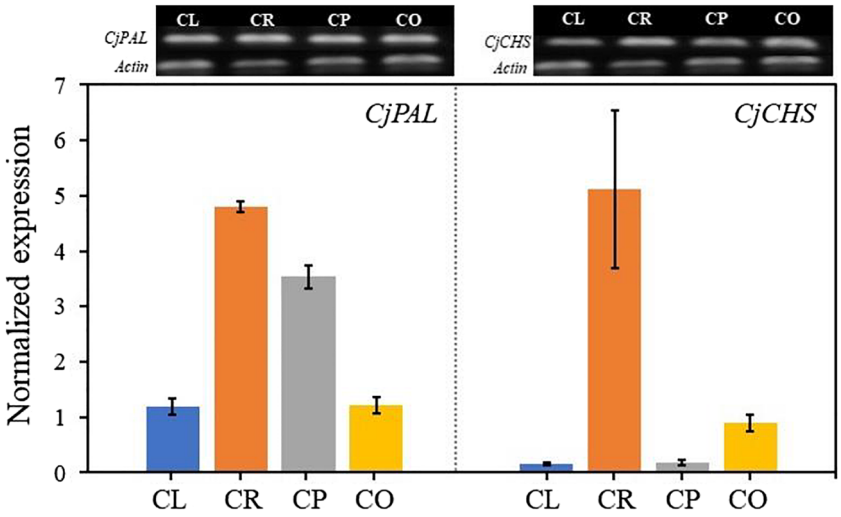

Figure 6. Expression of *CjPAL* and *CjCHS* in *C. japonica* calli derived from 4 different tissue origins after 4 wk of culture under RGB (red1/green1/blue) light (CL leaf-derived callus, CR root-derived callus, CP petal-derived callus, CO ovary-derived callus).

that the effects of circadian rhythms differ from organ to organ in *Arabidopsis*, and Thain *et al.* (2002) found that the expression levels of *CHS* differ in response to differences in the circadian rhythms associated with the leaves and roots of *Arabidopsis*. Therefore, we assume that there were differences in the expression of *PAL* and *CHS* observed in the present study when calli induced from different organs were cultured under illumination with a mixture of RGB light.

Consistently, we observed that total phenol and flavonoid content was the highest in CR callus (Fig. 7), which was characterized by the highest expression of *PAL* and *CHS*. High phenolic content was also detected in CP callus (5.66 mg g^{-1} DW), which we assumed that it is influenced by the expression of other downstream genes during phenolic biosynthesis. Moreover, along with CO (49.1%), we also found that DPPH radical scavenging activity was higher in CR (51.5%) callus under RGB light compared with that in the other two callus types (Fig. 7*b*).

In total, we detected 22 phenolic compounds, among which high levels of myricetin and physcion were observed in all lines (Fig. 8*a*). In terms of total phenolic content, CL and CR showed the highest levels (Fig. 8*a*). Levels of salicylic acid and cinnamic acid were found to be higher CL, CR, and CP calli, whereas (+)-catechin was detected only in CL callus (Fig. 8*a*). The 22 phenolic compounds we identified can be classified into five groups (hydroxybenzoic acid, hydroxycinamic acid, falvonols, anthraquinones, and other phenolic compounds), among which the content of flavonols and anthraquinones was higher in CL and CR calli (Fig. 8*b*), whereas no hydroxybenzoic acid was detected in CO callus. In all four callus lines, however, the content of flavonols was found to be higher than those of the phenolics in other groups. Myricetin is one of the major flavonoids found in numerous plant species and is known to have antioxidant, antitumor, anticancer, antiaging, and antiinflammation properties (Jung *et al.* 2010), whereas physcion is an anthraquinone derivative that plays a role in hepatocyte defense, and has antiinflammatory and antimicrobial activities (Wijesekara *et al.* 2014). In a comparison of the phenolic content of the roots, leaves, and stems in tea plant, Jiang *et al.* (2013) observed that the amounts of flavonol derivatives were the highest in the leaves, whereas those of proanthocyanidins were the highest in the roots. Thus, as indicated earlier,

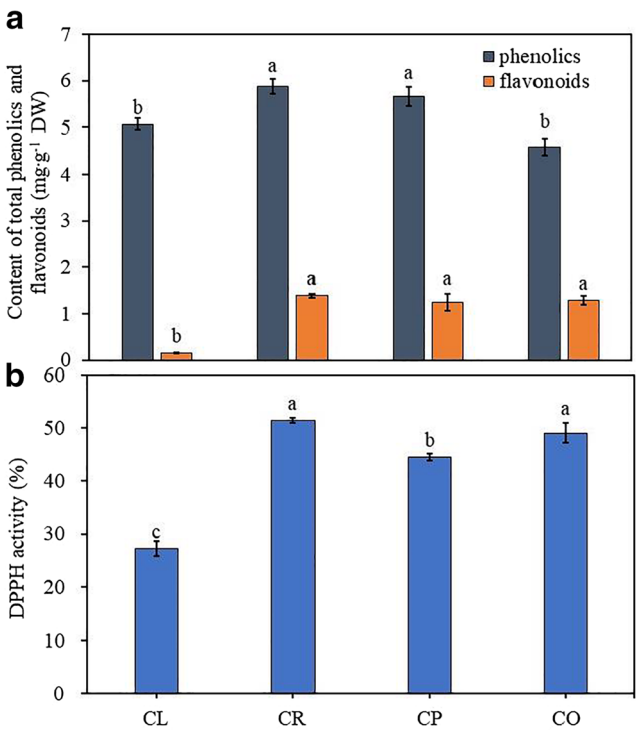

Figure 7. Antioxidant activity in *C. japonica* calli derived from 4 different tissue origins after 4 wk of culture under RGB (red1/green1/blue) light. (***a***) Content of total phenolics and flavonoids. (***b***) Free radical scavenging using DPPH (CL leaf-derived callus, CR root-derived callus, CP petal-derived callus, CO ovary-derived callus). *Different letters* indicate significant differences at $P < 0.05$ according to Duncan's multiple range test ($n = 3$).

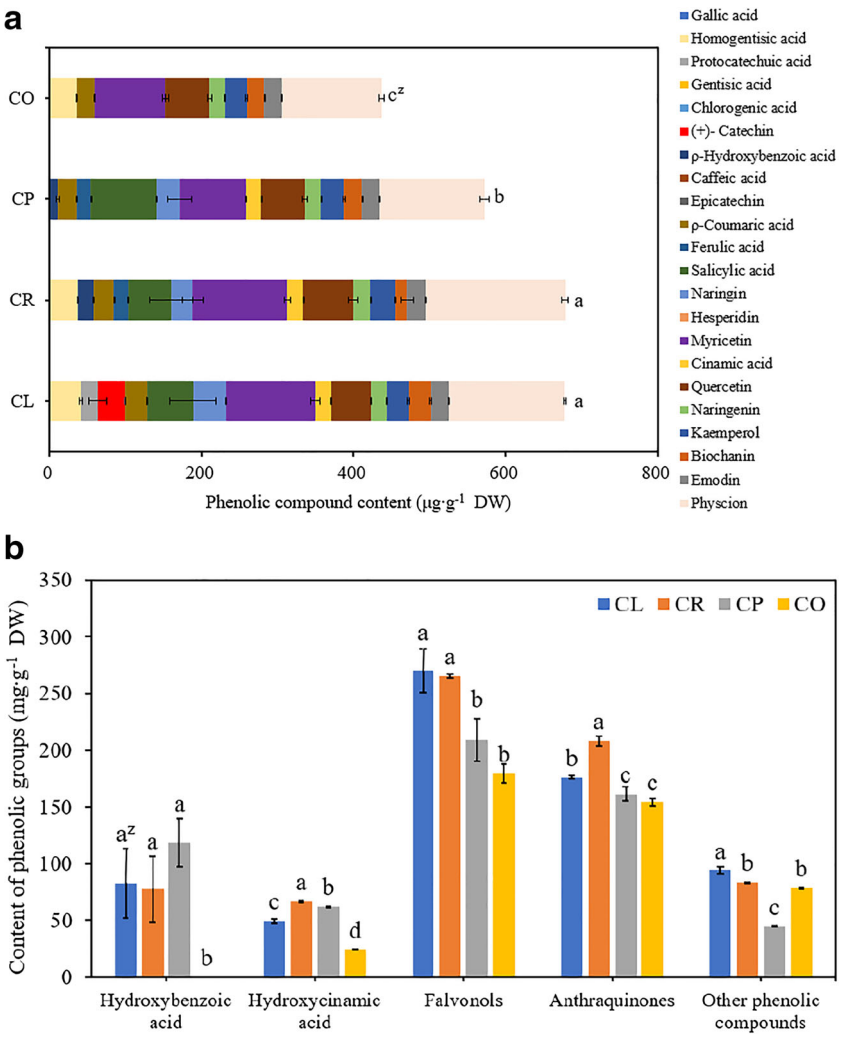

Figure 8. Phenolic profiling of *C. japonica* calli derived from 4 different tissue origins after 4 wk of culture under RGB (red1/green1/blue) *light.* (***a***) Content of specific compounds. (***b***) Content of phenolic groups (CL leaf-derived callus, CR root-derived callus, CP petal-derived callus, CO ovary-derived callus). *Different letters* indicate significant differences at $P < 0.05$ according to Duncan's multiple range test ($n = 3$).

differences with respect to the light sensitivity and circadian rhythms of different plant organs may contribute to differences in the types and amounts of phenolics detected in calli derived from these organs.

Conclusion

In this study, we investigated the effect of light quality and callus origin on the biosynthesis of phenolics in camellia callus culture. Our observations indicated that calli exposed to mixed light sources (RB and RGB) were characterized by high levels of both total phenolic compounds and DPPH radical scavenging activity. In addition, total phenol and flavonoid content and antioxidant activity were high in root- and petal-derived calli.

Author Contribution EB Jang contributed to the data acquisition and wrote the manuscript. TT Ho participated in the experiment and sample analysis. S-Y Park made substantial contributions to data interpretation, revising of the manuscript, the conception, and design of the study.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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